**Title**

The role of the methyltransferase SETDB1 in Chronic Kidney Disease

**Background**

Chronic Kidney Disease (CKD) is characterised by the expression of aSMA from the aberrant infiltration of myofibroblasts. This results in the deposition of ECM proteins, replacing normal kidney tissue and causing fibrosis, which subsequently leads to organ failure.

TGFβ1 has been found to play a major role in the persistent activation and proliferation of fibroblasts leading to fibrosis.

TGFβ1 signals through transmembrane serine-threonine kinase type I and II receptors to phosphorylate SMAD3, a downstream intracellular receptor-regulated SMAD protein (R-SMAD). Upon phosphorylation, activated SMAD3 translocates to the nucleus to regulate the transcription of fibrotic genes such as αSMA and collagen. Studies have shown SMAD3 to have an important role in fibrosis, SMAD3 null mice are resistant to tubulointerstitial fibrosis and glomerular fibrosis.

ERG-associated protein with SET domain (SETDB1), a methyltransferase, has been shown to mediate histone H3 lysine-9 (H3K9) trimethylation, gene silencing and transcriptional repression. H3K9 trimethylation is an epigenetic modification associated with transcriptional repression by recruiting HP1 proteins (amongst other transcriptional factors) to methylated histones.

Here we identify the involvement of the methyltransferase SETDB1 in the TGFβ1/SMAD3 pathway in relation to renal fibrosis.

**Methods**

**Luciferase Screen.** 3 pooled siRNAs targeting 48 known methytransferases were transfected into human renal epithelial cells stably transfected with the TGFβ1 reporter pCAGA12-luc. Controls were untransfected cells (UT), scrambled siRNA (SCR) and SMAD3 siRNA with or without 1ng/ml TGFβ1. The cells were transfected with 25nM methytransferase siRNAs then treated with 1ng/ml TGFβ1 for 24h.

**Immunoprecipitation.** Human renal epithelial cells were starved in serum free medium for 36h then treated with or without 10ng/ml TGFβ1 for 48h. Immunoprecipitation of SETDB1 was performed on nuclear fractions.

**Immunofluorescence.** Human renal epithelial cells were starved in serum free medium for 36h then treated with or without 10ng/ml TGFβ1 for 48h then subjected to dual-immunofluorescence using SMAD3 and SETDB1 primary antibodies and Alexa Fluor 568 (red) and 488 (green), respectively.

Human primary renal fibroblasts were transfected with either a SCR control siRNA or SETDB1 siRNA at 40nM concentration then treated with 10ng/ml TGFβ1 for 48h and subjected to immunofluorescence staining for aSMA primary antibody then Alexa Fluor 488.

**Results**

A siRNA library screen targeting human methyltransferases was undertaken to identify potential methyltransferases involved in TGFβ1/SMAD3 signalling. Resulting luciferase activity derived from the TGFβ1 driven reporter presented SETDB1 as a significant corepressor of SMAD3.

Immunoprecipitation of SETDB1 to dynabeads resulted in co-immunoprecipitation of SMAD3 from nuclear fractions of human renal epithelial cells in the presence of TGFβ1 treatment.

Human renal epithelial cells were starved then treated with or without TGFβ1 for 48h then subjected to dual-immunofluorescence, staining with SMAD3 and SETDB1. Upon TGFβ1 stimulation both SMAD3 and SETDB1 translocate to the nucleus demonstrating co-localisation.

Human primary renal fibroblasts were transfected with either a SCR control siRNA or SETDB1 siRNA and treated with TGFβ1 then subjected to immunofluorescence staining for αSMA. Upon SETDB1 silencing αSMA expression is increase compared to control siRNA.

**Conclusion**

We have demonstrated that SETDB1 translocates to the nucleus and interacts with SMAD3 in the presence of TGFβ1. Silencing SETDB1 in the presence of TGFβ1 increases the expression of αSMA. Our data suggests that SETDB1 is acting as a corepressor of SMAD3.